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Revision of the Chiapan deer mouse, *Peromyscus zarhynchus*, with the description of a new species

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We analyzed morphometric and molecular variation among 8 populations of *Peromyscus zarhynchus* grouped into 5 pooled samples representing separate physiographic regions across the range of this species in Chiapas, Mexico, and western Guatemala. Mitochondrial sequence data identify 2 well-supported and reciprocally monophyletic clades, separating all Chiapas specimens from those in Guatemala. These 2 clades group as a strongly supported monophyletic lineage aligned with other members of the *Peromyscus mexicanus* species group. The Chiapas clade is further subdivided into 4 subclades: 1) samples from the western part of the state, 2) specimens from a single locality in Northern Chiapas, 3) all central localities, and 4) those from a single locality in Eastern Chiapas. The molecular distance in the mitochondrial cytochrome-*b* gene (*Cytb*) between the 2 major clades is relatively low (mean *p*-distance = 3.66%); those between the 4 Chiapas subclades are even less (mean *p*-distance 2.73%). Multivariate analyses of external and craniodental morphometric variables also distinguish 2 major groups, separating Guatemalan from Chiapas samples but with the latter also divided into 2 subgroups, one that segregates the Northern Chiapas sample from those distributed elsewhere in that state. The Guatemalan and Chiapas samples differ in both cranial size and shape variables. The second-level separation of samples from within Chiapas (northern versus all others) is interpreted to result from the combination of local adaptation to distinct physiographic regions and geographic isolation generated by patches of suitable habitat. We describe the Guatemalan samples as a distinct species based on their molecular and morphological uniqueness, and argue that *P. zarhynchus* itself is divided into definable subspecies, with the nominotypical form *P. z. zarhynchus*, restricted to the vicinity of its type locality (Tumbalá) in Northern Chiapas, and *P. z. cristobalensis* with type locality of San Cristobal, over the remainder of the species range in the state.

Analizamos la variación morfométrica y molecular en 8 poblaciones de *Peromyscus zarhynchus*, agrupadas en 5 diferentes regiones fisiográficas a lo largo de la distribución de la especie en Chiapas, México, y el oeste de Guatemala. Los datos de secuencias mitocondriales identifican 2 clados monofiléticos recíprocos que separan a todos los individuos de Chiapas de aquellos de Guatemala; estos dos clados están agrupados como un linaje monofilético junto a otros miembros del grupo *Peromyscus mexicanus*. El clado de Chiapas está además subdividido en 4 subclados geográficos: (1) oeste, (2) norte, (3) todas las localidades del centro, y (4) este. La distancia molecular en el gen mitocondrial citocromo b (*Cytb*) entre los 2 clados principales es relativamente baja (distancia media p = 3.66%); mientras que entre los 4 subclados de Chiapas es aún menor (distancia media p = 2.73%). Los análisis multivariados de las variables externas y morfométricas también distinguen 2 grandes grupos, separando a las muestras de Guatemala de las de Chiapas; esta última es divisible en 2 subgrupos, uno con la muestra del norte de Chiapas y el segundo con aquellos distribuidos en otras partes del estado. Las muestras de Guatemala y Chiapas difieren en tamaño y forma craneal. El segundo nivel de separación de las muestras de Chiapas (norte contra todos los demás) es interpretado como resultado de la combinación de adaptación local a distintas regiones fisiográficas y aislamiento geográfico generado por parches de hábitat adecuado. Describimos a los ejemplares guatemaltecos como una especie distinta basada en su singularidad molecular y morfológica, y argumentamos que *P. zarhynchus* en sí mismo es divisible en subespecies definidas,

con la forma nominotípica (*P. z. zarhynchus*) restringida a los alrededores de su localidad tipo (Tumbalá) en el norte de Chiapas y *P. z. cristobalensis*, con localidad tipo en San Cristóbal, para el resto de la distribución de la especie en el estado.

Key words: Chiapas, Guatemala, Mexico, morphometric variation, *Peromyscus* nov. sp., *Peromyscus zarhynchus*, Rodentia

Peromyscus zarhynchus, the Chiapan deer mouse, is a member of the *Peromyscus mexicanus* group (sensu—Carleton 1989; Rogers and Engstrom 1992; Bradley et al. 2007), which also includes with certainty *P. grandis*, *P. guatemalensis*, *P. gymnotis*, *P. mexicanus* (sensu lato), and *P. nudipes* among species of deer mice currently recognized (see Pérez-Consuegra and Vázquez-Domínguez 2015). Morphological differentiation among all members of the *mexicanus* group is minimal and general genetic differentiation among them has been explained by geographic distance (Ordoñez-Garza et al. 2010). Within *P. zarhynchus*, however, subtle morphological and morphometric differences characterize samples from separate geographic areas throughout its limited range, particularly attributes such as tail color, dorsal fur length, hind foot color, caudal scale size and appearance, and overall cranial size. These differences are associated with local vegetation types and climatic conditions that exist across the species' distributional range (Lorenzo et al. 2006). Merriam (1898) distinguished 2 subspecies, describing *cristobalensis* (from San Cristobal in the central plateau of the Mexican state of Chiapas) in addition to the nominotypical *zarhynchus* (from Tumbalá in the northern mountains of that state). Current taxonomy, however, regards the species as monotypic (e.g., Huckaby 1980; Hall 1981; Musser and Carleton 2005). We consider the use of phylogenetic species concept as the proper term for defining them, since the 2 species maintain their identity from other lineages, possess their own historical and evolutionary trends, and have little gene flow because of their genetic isolation by geographical or biological barriers (Wiley 1978).

Peromyscus zarhynchus is currently considered endemic to the state of Chiapas, Mexico, ranging in elevation between 1,400 and 2,900 m (Romo and Horváth 2005) in cloud and pine-oak forest in northern and southeastern mountainous regions (Musser and Carleton 2005). In their general review of the species, McClellan and Rogers (1997) mapped only 3 localities, but more recent collections extend the range over a broader area of the northern mountainous region and central plateau of Chiapas, where the species has now been documented at 6 sites, and southeastward to near the Guatemala border in Lagos de Montebello National Park (see Supporting Information S1; Horváth and Navarrete 1997; McClellan and Rogers 1997; Lorenzo et al. 2006). Pérez-Consuegra and Vázquez-Domínguez (2015), however, provided molecular evidence for the presence of *P. zarhynchus*-like animals in western Guatemala in areas close to the border with Chiapas in their analysis of clade structure within the *mexicanus* complex. While these authors recommended species-level status for several named forms currently relegated as synonyms by others (e.g., Huckaby 1980; Musser and Carleton 2005), they did not comment specifically on the systematic status of these Guatemalan samples.

In central and Eastern Chiapas, *P. zarhynchus* is associated with primary cloud and pine forests, as well as disturbed secondary forests of each, vegetation communities dominated by *Pinus* sp., *Quercus* sp., and *Liquidambar* sp. All known localities are humid areas where rainfall peaks in the summer and temperatures are relatively cool (temperature averages over the 24-h period year-round of 18°C). In Western Chiapas, the species is associated with highland, very humid but warmer pine forests (mean annual temperature = 22°C—INEGI 1982). The habitat and climatic characteristics that define the range of *P. zarhynchus* in Chiapas also extend across the border into Guatemala, and the easternmost Chiapas locality currently known in Lagos de Montebello National Park is close to that border.

Herein we examine specimens of *Peromyscus* recently collected in Guatemala adjacent to Chiapas as well as elsewhere in Chiapas and review their systematic status relative to topotypic *P. zarhynchus*. The Guatemalan animals are the same as those examined in the broader molecular study of the *mexicanus* group by Pérez-Consuegra and Vázquez-Domínguez (2015). As we demonstrate, the specimens examined the diagnostic characteristics of typical *P. zarhynchus* but differ in overall size and in several qualitative cranial traits and, especially for the Guatemalan samples, separate strongly in both mitochondrial DNA sequence and multivariate morphometric analyses of craniodental variables. We conclude by defining a new species of deer mouse, from Guatemala and provide support for Merriam's original concept that *P. zarhynchus* is polytypic.

Materials and Methods

Available samples.—We examined a total of 289 specimens (163 males, 99 females, and 27 sex unknown) of presumptive *P. zarhynchus* from 15 localities, 9 in Chiapas and 6 closely adjacent sites in Guatemala. The entire sample was used in our morphometric analyses of external and craniodental traits, and a subset of 103 individuals were sequenced for a molecular phylogenetic perspective. For all analyses, we grouped these localities into 5 geographic units, 4 in Chiapas and 1 in Guatemala, based on both proximity and commonality of physiographic association: 1) Northern Chiapas (Tumbalá, the type locality), 2) Western Chiapas (Rayón and Coapilla), 3) Central Chiapas (Tzontehuitz, Oxchuc, and Huitepec Ecological Reserve), 4) Eastern Chiapas

(Lagos de Montebello National Park), and 5) Guatemala (Huehuetenango). We map each locality and identify the geographic groups in Supporting Information S1, and provide locality and specimen details in the Supporting Information S2. Voucher specimens are housed in the Colección Mastozoológica de El Colegio de la Frontera Sur, San Cristóbal de Las Casas, Chiapas (ECOSUR); Colección Nacional de Mamíferos, Instituto de Biología, UNAM, Mexico City (CNMA); Colección Zoológica Regional Mammalia, Secretaría del Medio Ambiente e Historial Natural, Tuxtla Gutiérrez, Chiapas (SEMAHN); Colección de Mamíferos del Museo de Historia Natural, Universidad de San Carlos, Guatemala (USAC); or Museum of Vertebrate Zoology, University of California, Berkeley, California (MVZ). All capture and handling methods followed the animal care and use guidelines of the American Society of Mammalogists (Sikes et al. 2011).

Molecular analyses.—We examined 3 different mitochondrial regions, including 2 functional genes (cytochrome *b* [*Cytb*; $n = 103$] and cytochrome *c* oxidase subunit III [*COIII*; $n = 51$]) and the nontranscribed control region (*CR*; $n = 96$). Specimens were sequenced from 13 of the 15 localities that comprise the 5 regional groups (Supporting Information S1 and S2).

We extracted genomic DNA from muscle or liver tissue preserved originally in either 95% ethanol or RNA later using the DNeasy Kit (QIAGEN, Inc., Valencia, California). We initially amplified an ~800 bp fragment of the *Cytb* gene using the primer pairs MVZ05 and MVZ16, with a subset subsequently amplified for the complete *Cytb* gene (1,143 bp) using primers MVZ127/MVZ14 and MVZ45/MVZ14 (primer sequences from Smith and Patton 1993; Leite and Patton 2002). We also amplified a 720 bp fragment of *COIII* using primers L8618 and H9323 (Riddle 1995) and a 995 bp *CR* fragment with primers H00651 and L16007 (Kocher et al. 1989).

We performed double-stranded amplifications using the following components and concentrations: 12.5 µL of template (10 ng), 4.4 µL ddH_2O, 2.5 µL of each primer pair (10 nM concentration), 0.474 µL (0.4 nM) dNTPs, 0.5 µL (3 mM) $MgCl_2$, 0.125 µL Taq polymerase (platinum, Invitrogen), and 1× Taq buffer to a final volume of 25 µL. Amplification conditions included 3 min of initial denaturation at 94°C followed by 37 cycles for *Cytb* and *COIII* and 40 cycles for *CR*; denaturation at 94°C for 45 s, 1 min annealing at 50°C for *Cytb* and *COIII* and 58.5°C for *CR*; and 1 min extension at 72°C. Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN) and templates were cycle-sequenced with primer pairs MVZ05/MVZ16, MVZ127/MVZ14, and MVZ45/MVZ14 for *Cytb*; L8618-H9323 for *COIII*; and H00651-L15926 for *CR*, using Big Dye terminator chemistry (Applied Biosystems Inc., Foster City, California). All sequences were run on an ABI 3730 sequencer (Applied Biosystems) at the Museum of Vertebrate Zoology. MVZ16, MVZ14, L15926, and L8618 were used to sequence the reverse strand in all individuals. All haplotypes from each of the 3 gene regions are deposited in GenBank (Supporting Information S3; accession numbers: *Cytb*—KT207314–KT207345, KU292362–KU292430; *COIII*—KT207297–KT207313, KU292485–KU292518; *CR*—KT207346–KT207378, KU292431–KU292484).

We aligned nucleotide sequences in Sequencher ver. 3.1 (Gene Codes Corp., Ann Arbor, Michigan), checked alignments by eye, and translated *Cytb* and *COIII* sequences to confirm the requisite lack of stop codons or gaps. Initially, we obtained sequences for an 800 bp fragment of *Cytb* from 113 individuals and then used TCS ver. 1.18 (Clement et al. 2000) to identify 32 unique haplotypes. From this set of unique sequences, we selected exemplar individuals from each of the 5 sample regions and obtained the complete *Cytb* gene (1,143 bp) from 43 specimens. TCS ver. 1.18 was also used to identify unique haplotypes for both the *COIII* and *CR* gene regions. We used Arlequin ver. 2.001 (Schneider et al. 2000) to estimate haplotype and nucleotide diversity for each grouped locality sample, as well as for the combined sample.

Phylogenetic analyses.—We employed both Bayesian and maximum likelihood (ML) approaches to estimate gene tree topologies. We determined the best substitution models for each of the 3 gene regions as well as for the concatenated series using the Akaike information criterion (AIC) as implemented in MrAIC (Nylander 2004). All analyses were performed with the unique sequences from each gene region, separately as well as on the concatenated dataset. Bayesian analyses were implemented in MrBayes ver. 3.1.1 (Ronquist and Huelsenbeck 2003), with 3 separate runs with Markov chain Monte Carlo simulations starting from a random tree, each with 3 heated and 1 cold Markov chains. Each run was conducted for 4,000,000 generations and sampled at intervals of 1,000 generations, with the first 1,000 samples of each run discarded as burn-in and all remaining sampled trees analyzed to find the posterior probability of resulting nodes. A consensus tree was generated with the 50% majority-rule algorithm in PAUP 4.0b10 (Swofford 2000), and the percentage of samples recovered in a particular clade was assumed to be the posterior probability of that clade. ML was performed in PAUP 4.0b10 using a heuristic search with 1,000 replicates and swapping with the TBR algorithm. We assessed reliability using each of the 3 codon positions separately, while applying equal weights, and nodal support using nonparametric bootstrapping. Since both Bradley et al. (2007) and Pérez-Consuegra and Vázquez-Domínguez (2015) demonstrated that *P. zarhynchus* is a member of the *P. mexicanus* species group, and the latter authors confirmed that our Guatemalan sample is sister to Chiapan *P. zarhynchus*, we used sequence data obtained from GenBank for the other members of this species complex: *P. grandis* (accession numbers: *Cytb*—KT308136–KT308137; *COIII*—KT308143–KT308144); *P. gymnotis* (accession numbers: *Cytb*—KT308132; *COIII*—KT308139; *CR*—KT308147); *P. melanophrys* (accession numbers: *Cytb*—KT308133; *COIII*—KT308140; *CR*—KT308148); *P. guatemalensis* (accession numbers: *Cytb*—KT308134–KT308135; *COIII*—KT308141–KT308142) as outgroups in all analyses. Finally, we summarized sequences divergence for each gene region among all pairs of geographic groups using uncorrected *p*-distances, which make no assumptions about substitution models.

Morphological analyses.—We obtained 4 conventional external measurements from specimen labels (total length [ToL], tail length [TaL], hind foot length [LHF], and ear

length [LE]), and took 19 linear measurements from cleaned skulls using digital calipers (0.01 mm resolution; measurements defined by Williams and Ramírez-Pulido [1984] and Robinson and Dippenaar [1987]). Craniodental characters included greatest skull length (GLS), skull height (SKH), condylobasal length (CBL), bullar length (BUL), shield-bullae depth (SBD), diastema length (DIL), rostral height (ROH), rostral breadth (BRR), palatine bridge length (PBL), postpalatal length (POL), basioccipital length (OCL), maxillary toothrow length (MTL), maxillary toothrow breadth (MTB), postdental breadth (PDB), zygomatic breadth (ZYB), braincase breadth (BAB), nasal length (NAL), interorbital breadth (IOB), and nasal breadth (NAB).

Following Hoffmeister (1951), we assigned all specimens to 1 of 3 age classes based on tooth eruption and wear patterns: juveniles (age class 1), subadults (age class 2), and adults (age class 3). We examined age and sex variation for all characters in 4 separate analyses, 1 including all specimens combined as well as in the 3 largest pooled samples from Central Chiapas (n = 154; 91 males, 63 females), Western Chiapas (n = 54; 37 males, 17 females), and Guatemala (n = 40; 19 males, 20 females, 1 unknown), using a least squares regression with both sex and age as covariates. To eliminate any effect of within sample variation due to sex and/or age on differences among samples, we computed residuals derived from the least squares regression for each variable. We then compared the results from subsequent analyses that used both the original variables and their residuals.

To examine character differences among the 5 geographic groups (see Supporting Information S1), we conducted 1-way analysis of variances (ANOVAs) for each of the 19 cranial variables. This analysis used the Tukey HSD (honestly significant difference) with unequal N (Spjotvoll/stoline) test to determine minimally nonsignificant geographic subsets. We also performed multivariate principal component analysis (PCA) and canonical variates analysis (CVA) to distinguish among specimens belonging to each physiographic region. Both multivariate analyses were implemented using log-transformations of the original variables. We excluded external measurements from our multivariate analyses because an unknown proportion of their variance is due to differences in preparator measuring methods. All statistical analyses were done using JMPPro (ver. 12.0.1, SAS Institute Inc., Cary, North Carolina—SAS Institute Inc. 2015).

Results

Molecular population variation.—The parameters of molecular diversity for each of the 3 gene regions and for both the separate geographic groups of *P. zarhynchus* as well as the combined sample are shown in Supporting Information S4. We also provide the mean number of pairwise differences for *Cytb* for each geographic group identified in Supporting Information S1. Not surprisingly, perhaps, both nucleotide and haplotype diversity scale somewhat with sample size, as those groups with the largest samples, have the highest values (Guatemala and Central Chiapas). The Eastern (n = 8) and Northern (n = 5) Chiapas samples had but a single haplotype and thus no diversity for each of the 3 gene regions.

Phylogenetic analysis.—The GTR+G (Tavaré 1986) model was selected by the AIC as the best-fit model of nucleotide substitution for each gene sequence separately as well as for the concatenation of all 3 (A = 0.3233, C = 0.2618, G = 0.1299, and T = 0.2850 for the latter), invariable sites = 0.0, Ti/Tv ratio = 4.03, and gamma distribution = 0.2584. AIC = 17140.75, K = 9, –Lnl = 8,561.38.

All haplotypes for each gene region and for the concatenated data are unique to a given geographic region, which are monophyletic with a posterior probability = 1 (see Supporting Information S5), regardless of whether the analysis was based on the concatenated or individual gene sequence data sets. All analyses also identified the same 2 major, reciprocally monophyletic clades (again with posterior probability = 1) within our *P. zarhynchus* sample, sharply separating the Guatemala sample from the 4 Chiapas ones. This topology is similar to that found with *Cytb* sequences in Pérez-Consuegra and Vázquez-Domínguez (2015), where the Guatemalan sample corresponds to "lineage G" and the Chiapas subclades Central and Western corresponds both with "lineage E" and Eastern subclade with "lineage F." Among the latter, further hierarchical subclades are apparent. First, the Eastern Chiapas sample, with its single, unique haplotype for all 3 genes, appears basal to those from the Central, Northern, and Western geographic groups, which themselves are further subdivided into Central versus Northern + Western. The presence of only a single haplotype in the Northern and Eastern samples precludes the ability to calculate support values separate from that linking each to their sister clade (see Supporting Information S5). The ML analysis with the GTR+G evolution model produced a single tree (score = 6,133). The topology and nodal support values (based on 100 bootstrap replicates) of this tree (not shown) were identical to those derived from the Bayesian analysis.

The specimens from Guatemala are 3.66–4.71% (p-distances) divergent from Chiapan samples for the concatenated gene sequences, and 3.66–4.38% for the complete *Cytb* dataset. Specimens from the 4 Chiapas subclades differ by an average of 3.09% in the concatenated analysis and range from 2.08 to 3.65% in their respective *Cytb* sequences (see Supporting Information S6).

Morphological comparisons.—Specimens from each of the 5 geographic groups (see Supporting Information S1) share similar morphological characters. Externally, the ears are large and almost naked, the hind feet are long and relatively narrow, the upper parts are dusky, under parts are whitish, the tail is dusky above and yellowish white below, and fore and hind feet are whitish with the latter slightly more gray dorsally. The color of dorsal and ventral pelage is dark, although the specimens from Guatemala appear darker than those from Chiapas.

Only a single craniodental variable exhibited sexual dimorphism in the total pooled sample (BUL; P = 0.0001). Furthermore, among the 3 geographic groups treated separately, sexual dimorphism was apparent only in the Central Chiapas sample (3 of 19 traits [CBL, OCL, and ZYB], all with P between 0.0492 and 0.034); all variables were monomorphic

in both the Western Chiapas and Guatemala samples. Age differences are more substantive, as might be expected, with significant differences, most with a *P*-value of 0.001 or below, for the majority of variables in each sample group (total pooled sample, 16 of 19 variables [only BRR, PDB, and NAB were nonsignificantly different among the 5 sample groups]; Central Chiapas, 12 of 19 variables [SKH, BUL, BRR, MTL, PDB, BAB, and IOB nonsignificant]; Western Chiapas, 15 of 19 variables [CBL, BRR, PBL, and PDB nonsignificant]; and Guatemala, 13 of 19 variables [SKH, MTL, PDB, BAB, IOB, and NAB nonsignificant]).

Geographic variation.—Means and *SE*s for each external and craniodental variable are given in Supporting Information S7, as are significance levels in comparisons across all 5 geographic sample groups based from 1-way ANOVAs. Data are provided for only full adults, or age class 3 individuals. Specimens from Northern Chiapas are similar to those from Eastern Chiapas in total length (these samples differ only by 0.33%), and both are, on average, larger in total length than those from Western Chiapas (3.24%), Central Chiapas (9.15%), and Guatemala (12.32%). Similarly, specimens from Northern Chiapas have both longer tails and hind feet relative to those from other geographic areas: Eastern Chiapas (3.95% and 6.81%, respectively), Western Chiapas (6.99% and 5.92%), Guatemala (4.80% and 2.96%), and Central Chiapas (14.04% and 9.47%). In contrast, Guatemalan specimens had longer ears (3.54% larger to those from Northern Chiapas, 4.73% larger than Eastern Chiapas, 8.27% larger than Western Chiapas, and 9.45% larger than Central Chiapas).

When analyses are restricted to adults of age class 3, only 3 of the 19 craniodental variables (SKH, MTB, and ZYB) are not statistically significant across the 5 pooled geographic samples by 1-way ANOVA (see Supporting Information S7). In a similar analysis constructed on the character residuals for these craniodental variables, which removes the potential confounding effects of sex and age differences, all variables exhibit highly significant intergroup differences, all but 3 (SKH, MTB, and ZYB) at $P < 0.0001$. Tukey post hoc tests (see Supporting Information S8) segregate geographic samples in 18 of 19 variables in the residual character dataset but in only 15 variables when the analysis is conducted only on age class 3 individuals. When limited to age class 3 individuals, the Eastern Chiapas sample has significantly longer CBL than other samples, (mean 35.16 mm versus < 31.56 mm) and the Northern Chiapas sample is separated from others with by longer OCL (6.22 mm versus < 5.5 mm) and MTL (6.32 mm versus < 5.47 mm). The Guatemala and Northern Chiapas samples share statistically similar BUL (5.43 and 5.40 mm, respectively) relative to other samples. And all 5 geographic groups have statistically unique PBL measurements. Other than these few, and largely unitary examples, no general pattern of character difference is apparent when all 5 groups are viewed together for any other characters. A regression of GLS, for example, on latitude is not significant (*F*-ratio = 0.1416, $P = 0.7278$), largely because the Central Chiapas sample is smallest (mean GLS = 36.65 mm; see Supporting Information S7) and the geographic extremes (Northern Chiapas and Eastern Chiapas) are largest (37.97 and 39.10 mm, respectively).

The first 4 factors of the PCA, each with eigenvalues > 1.0, explained 63.48% of the total variation among 19 cranial characters: PC1 = 31.84%, PC2 = 17.50%, PC3 = 8.12%, and PC4 = 6.02%. Loadings on PC1 are positive, suggesting that this axis represents general size, but with GLS (eigenvector 0.361), DIL (0.341), POL (0.326), ZYB (0.321), and ROH (0.315) contributing equally strongly to the dispersion of individuals on that axis. Scores on PC2 largely contrast BUL (0.396), PDB (0.338), and BAB (0.321) with CBL (−0.399) and BRR (−0.366); those on PC3 separate specimens primarily by SKH (0.492) and NAB (0.470) versus SBD (−0.330); and those on PC4 contrast MTL (0.483) with PBL (−0.516), PDB (−0.398), and MTB (0.345). In individual scores by post hoc ANOVA tests of differences among the 5 geographic groups, there is statistical significance to the group-specific PC1 scores, but there is no geographic pattern to those "size" differences. The geographic groups, however, can be distinguished by their respective scores on each of the "shape" axes (PC2 to PC4). For example, PC2 scores group Guatemala and Northern Chiapas samples together separate from the other 3 Chiapas samples; Guatemala and the Central Chiapas sample, while separate from one another, are distinguished from other Chiapas samples by their PC3 scores; and, similarly for PC4 scores, both the Northern Chiapas and Guatemala samples, each separate from one another, are likewise distinguished from remaining Chiapas groups (see Supporting Information S8 for summary of Tukey post hoc test comparisons).

Canonical variates analysis performed on the 5 sample groups separates Guatemala and Chiapas samples on the 1st axis (CV1; see Supporting Information S9) and the Northern Chiapas sample from the remainder from that state on CV2. CBL and PBL had contrastingly high loadings on CV1, with POL also contributing to separation along this axis (see Supporting Information S10; see inset, Supporting Information S9) The 2nd CV axis separates the Northern Chiapas sample from the Western, Central, and Eastern Chiapas groups, with negative values for CBL, SBD, and NAL versus positive values for OCL and ROH contributing most strongly. The 2 Chiapas groups identified on CV2 include topotypes of *zarhynchus* Merriam (Tumbalá, Northern Chiapas sample) and near-typotypes of *cristobalensis* Merriam (Cerro Tzontehuitz, near San Cristobal, Central Chiapas group), respectively.

All individuals of both the Guatemala and Northern Chiapas samples were correctly classified to their respective groups, but only 85% for specimens from Central Chiapas (125 of 147), 95% for those from Eastern Chiapas (19 of 20), and 81.5% for those from Western Chiapas (44 of 54) were so classified. All misclassified individuals were assigned to another of these 3 geographic groups, with misclassified nearly evenly divided between the other 2 groups in the Central and Eastern Chiapas samples with the largest sample sizes. Overall, the multivariate pattern of craniodental variation among a priori defined groups mirrors the molecular clade structure depicted in Supporting

Information S5, particularly and importantly in the separation of a Guatemala clade that is basal to all Chiapas clades.

Taxonomic Implications

The *P. zarhynchus* samples we examined are divided into 2 strongly supported and reciprocally monophyletic clades (see Supporting Information S5), one distributed throughout Chiapas and the other in adjacent Guatemala (see Supporting Information S1). While these clades are allopatric, they approach one another, with the Eastern Chiapas sample only about 25 straight-line km from the Guatemalan localities (see Supporting Information S1). And, although clade structure with such a high level of support is consistent with the Phylogenetic Species Concept (Cracraft 1997), the level of *Cytb* divergence, for example, between them averages only 4.27%, a value lower than the species-level threshold posited by the Genetic Species Concept (Bradley and Baker 2001). The morphological distinctness of members of the 2 clades in both size and cranial shape parameters (see Supporting Information S9), however, is striking. We believe that the combined morphological and molecular distinctness justify our recognition of the Guatemalan specimens as a species, which we describe below.

The multivariate morphometric analyses further demonstrate morphological separation of specimens from Northern Chiapas from the 3 other samples in the state. This distinction supports the earlier view of Merriam (1898) when he delineated 2 subspecies of *P. zarhynchus*, the nominotypical *P. z. zarhynchus* from Northern Chiapas (with type locality of Tumbalá) and *P. z. cristobalensis* from the remainder of the species range (and with type locality of San Cristobal). In comparison to *P z. cristobalensis*, *P. z. zarhynchus* has a duskier dorsum, becoming seal brown and orange on the sides, and a less whitish venter with the pectoral region washed with chestnut, the chestnut suffusion usually spreading over the belly; the tail is dusky above, yellowish white below; fore and hind feet are less whitish, the latter clouded.

Peromyscus gardneri sp. nov.

Holotype.—USAC 4759/MVZ 223293 an adult male collected by Sergio G. Pérez (original number 1512) on 9 January 2009, at Guatemala: Finca Ixcansán, 10.3 km E (por carretera) Aldea Yalambojoch, San Mateo Ixtatán, Huehuetenango, 1,647 m, 16.00614°N, 91.49988°W; physiographic region 5 in Supporting Information S1). The specimen consists of a stuffed museum study skin with accompanying cranium and mandibles (see Supporting Information S11) housed at USAC, and liver tissue preserved in 95% ethanol with aliquots maintained frozen at both USAC and MVZ; all parts are in good condition.

Paratypes.—USAC 4746, 4748–4750, 4756, 4761–4764, 4790, 4791; and MVZ 223228–223305 (see Supporting Information S2 for localities).

Common name.—Gardner's deer mouse.

Etymology.—We honor Dr. Alfred L. Gardner for his long and distinguished career in the mammalogy of Mexico, Central, and South America; his expertise has contributed substantially to our understanding of the systematics, taxonomy, and wildlife biology throughout the Neotropics, especially through his direct training of scholars in the field and museum and by his set of exemplary standards that we, and others, can only hope to emulate.

Nomenclature statement.—A life science identifier (LSID) number was obtained for the new species *Peromyscus gardneri*: urn:lsid:zoobank.org:pub:AC01BC8C-218E-4077-91C2-694C835AC0F7.

Diagnosis.—A member of the *P. mexicanus* group, *P. gardneri*, is characterized externally by a large body, large ears, long tail, and moderate length hind feet; cranially by a long skull, maxillary toothrow, auditory bulla, basioccipital, and mandible contrasting with a diastema of moderate and enlarged orbits (see Supporting Information S7 for comparisons), and especially the absence of prominent supraorbital ridges that define the "beaded interorbital region" characterizing other members of the *mexicanus* group.

Description and comparisons.—*Peromyscus gardneri* differs externally from *P. guatemalensis*, a mouse that also inhabits the Sierra de los Cuchumatanes of Guatemala but at higher elevations, by larger size (mean total length 265.4 mm for *P. gardneri* versus 259 for *P. guatemalensis*), longer ears (mean 25.4 mm for *P. gardneri* versus 21.4 for *P. guatemalensis*), a darker and wider middorsal stripe, a blotchier pattern of dark gray and white patches on the venter, a higher frequency of a prominent orange patch on the chest, and a ventral tail surface that is dark at its base but becomes blotchy distally. Cranially, *P. gardneri* is distinguished from *P. guatemalensis* by a larger mandible and molar teeth, an especially more pronounced mandibular condyle, a wider mandibular masseter area, a wider squamosal at the base of zygomatic arch, and, in the postcranial skeleton, an innominate with a shorter pubis and ischium and a pronounced pubic arch, which is much smaller or absent in *P. guatemalensis*.

Both *P. gardneri* and *P. zarhynchus* share most of the characters noted above, but *P. gardneri* has a more robust mandible (mean mandible height 8.3 mm for *P. gardneri* versus 8.2 mm for *P. zarhynchus*), and slightly smaller molars (mean maxillary toothrow length 5.5 mm for *P. gardneri* versus 5.6 mm for *P. zarhynchus*), a relatively longer rostrum, and both a wider and shorter incisive foramina that do not reach posteriorly to the level of the molars. The lower jaw of *P. gardneri* has a straight, rather than concave, and less pronounced angular process, but a wider mandibular condyle and slightly longer and rounded coronoid processes. With respect to *P. z. cristobalensis* (from San Cristobal), *P. gardneri* is slightly larger with longer ears, a longer tail that is nearly naked, and long hind feet that appear narrower and with darker surfaces over the metatarsals but with white digits. The dorsum is darker, the venter is grayish, and the lateral orange stripe is less obvious. With respect to *P. z. zarhynchus* (from Tumbalá), *P. gardneri* is slightly smaller on average but has larger ears, a shorter and nearly naked tail, and shorter hind feet that are less slender and darker, but also have white digits. The dorsum is darker but the venter is of the same white intensity, and the lateral orange stripe is of

equivalent development. The external and craniodental values for the holotype as well as the mean and range for male and females paratypes are shown in Supporting Information S12.

Distribution.—Peromyscus gardneri is currently known only from 6 midelevation sites (approximately 1,000–1,700 m) on the northern slope of the Sierra de los Cuchumatanes in western Guatemala, a karst topographic region covered by humid cloud forest. This mouse is likely endemic to the Sierra de los Cuchumatanes, probably limited to the south by the cold pine and pine-oak forests that characterize the higher elevations of the range, to the west by lowland, warm tropical rain forest, and to the east by the large canyon of the Río Ixcan. Northern limits along the México–Guatemala border are less clear, but are likely affected by the presence of a series of midelevation pine forests.

Ecology.—Peromyscus gardneri is an abundant mouse, numerically the most dominant species over its known distributional range, a region where precipitation is among the highest in Central America. All specimens were captured on the forest floor in dense but highly degraded forest, principally near hollows at the base of trees or rocks that are common in this karst topography. Diet, based on stomach contents from 5 individuals, included stems from small plants, small fruits (exocarp skins about 1 cm in diameter, mesocarp mash, and small oval seeds about 2 mm long), and adult orthopterans and coleopteran body parts. Stomach morphology is the bilocular-discoglandular type identical to that described and figured for other members of the *mexicanus* group, with the glandular epithelium occupying an almost completely closed pouch located on the greater curvature (Carleton 1973:20, figure 9D). Seasonal reproduction activity is unknown, but our type sample, all collected early in the month of January, included juveniles, subadults molting into their adult pelage, and adult individuals. Among the latter (defined as specimens in adult pelage, with slightly to well-worn molars, and a body mass > 70 g), only 1 out of 8 males autopsied had scrotal, enlarged testes (testis length × width 16 × 11 mm, compared to a mean testis size of 8 × 4 mm for nonscrotal males) and all females displayed placental scars, indicative of prior breeding efforts, but none were pregnant. Scar counts for 1 female were 3 in each uterine horn, suggesting a litter size of 6 neonates. Given these limited data, reproduction took place at least in the immediately prior fall months but had ceased by the time of our visit to the area in early winter.

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Supporting Information

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Population samples of *Peromyscus zarhynchus* complex from Chiapas, Mexico, and *P. gardneri* from Huehuetenango, Guatemala; samples are pooled by physiographic regions and geographic proximity: 1) Northern Chiapas, 2) Western Chiapas, 3) Central Chiapas, 4) Eastern Chiapas, and 5) Guatemala (see text and Supporting Information S2). Letters indicate physiographic regions: A) Mountains of Northern Chiapas and B) Central Plateau of Chiapas. Dotted lines indicate the distribution of both species.

Supporting Information S2.—Specimens examined. The 289 specimens examined are listed below by geographic group, as identified in Supporting Information S1, with locality and museum acronym plus catalog number provided. For those specimens sequenced, the genes are indicated following each catalog entry. Collections and their acronyms are Colección Mastozoológica de El Colegio de la Frontera Sur (ECOSUR); Colección Nacional de Mamíferos del Instituto de Biología de la UNAM (CNMA); Colección Zoológica Regional Mammalia, Secretaría del Medio Ambiente e Historial Natural (SEMAHN); Colección de Mamíferos del Museo de Historia Natural de la Universidad de San Carlos, Guatemala (USAC); and University of California, Berkeley, Museum of Vertebrate Zoology (MVZ).

Supporting Information S3.—Specimens examined for molecular data. The 103 specimens examined are listed below by geographic group, as identified in Supporting Information S1, with locality, GenBank accession numbers, and museum acronym plus catalog number provided. Locality, collections, and their acronyms to each specimen are given in Supporting Information S2. The genes are cytochrome *b* (*Cytb*), cytochrome *c* oxidase subunit III (*COIII*), and control region (*CR*). Specimens with the complete *Cytb* (1,143 bp) are marks with asterisk. Haplotypes in italic are used in the Bayesian analysis.

Supporting Information S4.—Sample size (*n*), number of haplotypes (*k*), haplotype diversity (*h*), nucleotide diversity (π), the number of polymorphic sites, and mean number of substitution differences obtained for the *Cytb*, *COIII*, and *CR* gene regions for each of the 5 geographic groups of *Peromyscus zarhynchus* (*sensu lato*) from Mexico and Guatemala, and for the combined sample dataset.

Supporting Information S5.—Bayesian tree derived from all haplotypes from the 5 geographic groups identified in Supporting Information S1. The tree is rooted with sequences from 4 other species in the *Peromyscus mexicanus* group that are currently recognized (see text and Bradley et al. 2007; Pérez-Consuegra and Vázquez-Domínguez 2015). Solid circles at nodes denote Bayesian posterior probabilities = 1 and maximum parsimony bootstrap values of 100. Terminal triangles are proportional to the number of unique sequences contained in that clade.

Supporting Information S6.—Matrix of mean uncorrected *p*-distances expressed as percentages between each pair of geographic groups, as identified in Supporting Information S1. Values above the diagonal are those for the 3 concatenated gene sequences; those below the diagonal are for the separate *Cytb*, *COIII*, and *CR* sequences, respectively.

Supporting Information S7.—Mean values for 4 external and 19 craniodental characters for adults (age class 3) of each of the 5 geographic groups of *Peromyscus zarhynchus* from Chiapas and Guatemala. *F*-values and significance levels for comparisons among samples by 1-way ANOVA are given. Character abbreviations are given in the text; *n* = sample sizes, *SD* = standard deviation.

Supporting Information S8.—Morphometric variation between 19 cranial variables of specimens of *P. zarhynchus* of 5 physiographic regions from Chiapas and Guatemala, according to a single-classification analysis of variance, and the Tukey *post hoc* test for both character residuals and age class 3 individuals. Regions not connected by same letter are significantly different ($P < 0.05$). EC = Eastern Chiapas; NC = Northern Chiapas; CC = Central Chiapas; WC = Western Chiapas; G = Guatemala.

Supporting Information S9.—Bivariate plot of scores for each geographic group of *P. zarhynchus* (sensu lato) for the first 2 canonical variates axes. Groups are identified by names (see Supporting Information S1) and symbols; small black circles indicate group centroids and ellipses bound peripheral specimen scores for each group. The inset box illustrates vectors for the 7 variables that load most highly on the 2 axes (see Supporting Information S10), and thus that influence the position of the a priori defined geographic groups on this pair of axes.

Supporting Information S10.—Standardized scoring coefficients (eigenvectors) for the first 3 canonical axes in the analysis of the residuals of 19 craniodental variables of *Peromyscus zarhynchus* (sensu lato).

Supporting Information S11.—(A) Dorsal and ventral views of the study skin and (B) dorsal, ventral, and lateral views of the skull and lateral view of the mandible of the holotype of *P. gardneri* (USAC 4759).

Supporting Information S12.—External and craniodental values for the holotype followed by the mean and range for male and female paratypes of *P. gardneri*.

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